March 8, 1953

Dear Dr. Felix:

I have delayed this reply to your letter of February 10 to complete some comparisons with various S. typhi O 901 subcultures, amid other tasks. Your package arrived airmail promptly on the 17th of February, just prior to my return from a three-weeks trip to Chamblee, Ga.

I have read your remarks on phage with the most attentive interest. Perhaps I could conclude a summary of my own position with the notion that while I cannot dispute your position, and am willing to accept it with only the reservation that the alternative views have still something to offer that may someday be reconciled with your own. Perhaps after having acquired more experience, and with this, confidence, in thinking about the problem I will be able to adopt a more affirmative perspective.

To turn again to more tangible problems, your two subcultures of O-901 have behaved much like the copies already examined here. That is, with the help of a sufficiently exacting selective technique, it is not difficult to secure motile reversions, all of which have been d:- (another example of the genetic stability of a latent potentiality). This finding is not inconsistent with the stability of O-901 in laboratory practice, and is quite unlikely to have any practical effect anywhere, as the "mutation rate" for restored motility would be negligible except under drastic selection. My technique has been to stroke loopfuls of broth cultures of O-901 on Petri plates of soft gelatin-agar (according to Edwards, etc). After 48 hours incubation, one will observe, in general, about one swarm to a plate which, of course, eventually spreads throughout the plate. Such swarms can be induced in very much greater numbers by the transduction of motility factors from other strains, but are always d, regardless of the serotype of the source strain. Dr. Stocker may have mentioned some more details of these experiments. O-901 is sufficiently stable, in our judgment, as to be useful in these experiments; many other "O-variants" have proved to be so much less stable as to permit confusion between spontaneous and transduced motility. I can only conclude that this plate method is more effective in selecting for motile reversions than the techniques more generally practiced, probably because of the much larger size of the population tested, for motile components. I am very glad to have your comment on preservation of cultures: we are adopting this technique forthwith. May I add that the "B-O" strain recommended for serum diagnosis, if this is the same as Edwards' #13, Ky. Bull. 54, 1942, is also susceptible to reversion though much more slowly and infrequently than O-901. There are few things absolutely stable under the sun, if we have the resources to examine sufficient numbers!

It is against the philosophical background of this statement that I venture to suggest that the Booy-Wolff & Landy experiments should be examined more fully. I am fully concordant with your criticism, and pleased that it issued from so authoritative a source, for I am sure that these phenomena are not what they appear, prima facie, to be to these workers. At least, Booy & Wolff and Crézé have not convinced me that they have induced any change, or transferred any trait. They do appear to have a technique by which, as with animal passage, Vi+ or Vi-like strains can be selected from O-901, a result which is not feasible by ordinary in vitro manipulations. It seems not unreasonable to me that the cooked bacteria used in these experiments may develop some antibacterial properties, against which the Vi substance serves for some protection. At any rate, I am sure that your criticism will help to spur a more thorough analysis of

the change.

Please forgive me for my obfuscation about "recommending Creze's address": I was rather passing on what Creze asked of me, which I gather is that I, in a sense, introduce him to you. At considerable risk of presumption, may I consider this done? I have been hoping that Creze could be induced to correspond with you: unlike some of your other correspondents, he appears to have hit upon the same phenomenon, but is rather eager not to jump to any conclusions about it, and to seek some experienced advice. On Vi questions, I obviously cannot do this, but it is some measure of Professor Creze's unfamiliarity with the field that he has not consulted you in the first instance. I imagine that he will write to you directly, on my recommendation. (His address, by the way, is Institut de Bacteriologie, Faculte de Medecine, Angers, France).

I am pleased to have a better understanding of your remarks on the role of O antigens as phage receptors. Your qualifications about widely overlapping specificities were clearly understood; I wondered, if there were not still some particular case where a receptor could be identified as a major O component. PLT-22, for example, does not appear to be adsorbed by any bacterium lacking the XII complex. The exceptional XII-containing bacteria that do not adsorb, paratyphi A (Edwards' # 1) and abortus-bovis (#28) were such as to suggest that the XII_2 fraction might be implicated. I have been seeking advice on other experience with these relationships, and on whether or how best to pursue them. I have a better appreciation of some of the difficulties (e.g. Edwards' finding that S. pullorum strains occur that evoke anti-XII_2 antibodies, but neither agglutinate in nor absorb them), and realize that it may be very difficult to clarify exceptional reactions. Your most recent reprints (for which my thanks) serve no greater encouragement. I am still not clear on your experience with "XII_1, XII_2, XII_3" as receptors for phages. Are there grounds for assigning some phages as specific for these components? Or is the classification of these components so violent an over-simplification as to vitiate any such enterprise?

As to Boulgakov's phage, I need have no concern as to its reliability of this laboratory (though, in fact, two of four samples so represented were not H phages at all). "VIII-13" appears to conform to Sertic's original description; its lack of H-specificity is based on further experiments which in no way contradict the original tests: they were simply not extensive enough.

I am still working with the phages O1-3. While as you say, they rarely sterilize a sensitive culture, one needs survival ratios of perhaps 1% or more for successful transduction. However, I believe I now have some resistant variants, still "smooth", which adsorb O3 without gross lytic response and these may be more amenable.

While working with Edwards last month, I tried to look into the question why some serums would evoke phase variants from so-called monophasic types, such as S. choleraesuis, kunzendorf. The ineffectiveness of kunzendorf and berlin, 1,5...-serums, as contrasted with the effectiveness of other 1,2 and 1,5... serums in securing the c phase has been thought to be possible evidence that these variations are induced as well as selected by the 1,5 serums. As one might expect, the result actually appears to depend on (unsuspected?) cross-reactions. The c phases that can be obtained from kunzendorf will agglutinate to 1:500 in berlin serum, but the reactivity is impaired not only by heat, as is usual for the H antigens, but also by the customary formalin treatment. This latter is why I imagine the reaction not to have been detected earlier (or has it in fact been recognized), and if so formalin-lability should perhaps be attended to in other investigations. (I have not yet been able to consult Edwards for this last detail, so please hold me responsible if it is a blunder). It hardly needs remark that this may be another source of confusion in unintelligent slide-agglutination, but must admit that this is how

perhaps more close attention can be given to the reaction was first detected. In general, what serums are used to detect alternative phases: while typhimurium 1,2,5/ serum is probably best for suspected kunzendorf strains/ isolated in 1,5 phase, it serves very poorly for javiana and "morehead 1,5..." serum may be much better.

Just an ad hoc suggestion from reading your recent reprints, on the behavior of bacteria heated at 75°. Might the peculiar physical behavior be related to the release of nucleic acids? Some workers in Italy have reported that these are liberated at, I think, about this temperature. It would not be difficult to test the effect of some desoxyribonuclease or ribonuclease on the consistency of the suspensions. (Cf. W.H.O. Bull. 6:... 1952: I don't have the full reference).

At Dr. Edwards' instigation, and with his help, we have managed to construct a Salmonella: IX XII 1,2:1,5, obtained by S. "java phase 2", Edwards' #157 --x S. miami. This serves only to emphasize the anomalous genetic behavior of #157. This may have something to do with the result (instigated by a comment from Spicer) that the original java phase 1, "B:--", agglutinates to over 1:200 (living cells) in anti-#157 serum.

Yours sincerely,

Joshua Lederberg